From Gene Expression modelling to CoRegulation networks for Arabidopsis

Rim ZAAG
PhD student
Bioinformatics for Predictive Genomics Team

Thesis supervisors
Marie-Laure Martin-Magniette
Etienne Delannoy

NETBIO
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Context and Background

- A huge ‘orphan of function’ gene space:
  - 20% to 40% of the predicted genes for completely sequenced eukaryotic organisms have no assigned function (Hanson et *al*., 2010).
  - More than 5000 Arabidopsis genes are still hypothetical or unknown genes according the TAIR v10 annotation.
- Our knowledge about gene candidates involved in the adaptation of plants to their environment remains partial (of potential interest for crop improvement).

Context and Background

- Functional annotation procedures based on sequence similarities have reached their limitations

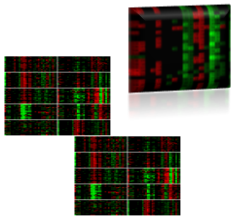
- One gene-one enzyme hypothesis is now considered as an oversimplification

- Availability of thousands of transcriptomes: allow us to shift from a 'gene by gene' approach to more global approach through the 'guilt by association' concept.

- **Hypothesis: Coexpressed genes have likely related biological functions** (Eisen et al., 1998)

Classical Flowchart

Gene Expression Omnibus



CoExpression analysis

Bioinformatic characterisation
(GO, TFBS, PPI,TF-target, bibliome…)

Focus on specific genes for biological validation

Drawbacks

- Data are generally extracted from international repositories
- It leads to heterogeneous data in terms of acquisition and preprocessing.
- Coexpression generally done by analyzing gene pairs (Pearson correlation)
- It is a local point of view of a complex question.

Our Approach: Goals & methods

Goals:

I. Provide a global overview of the coexpression units of genes responding to a panel of stress stimuli in *Arabidopsis thaliana*.
II. Go beyond the coexpression to identify coregulated modules of functional partner genes.
III. Inference of function to orphan genes in well-characterized modules.

Methods: original features

- The specificities of the dataset and the biological theme: homogeneous and dedicated transcriptomic data.
- The method of clustering: model based method
- The integration of various resources to improve the functional inference.

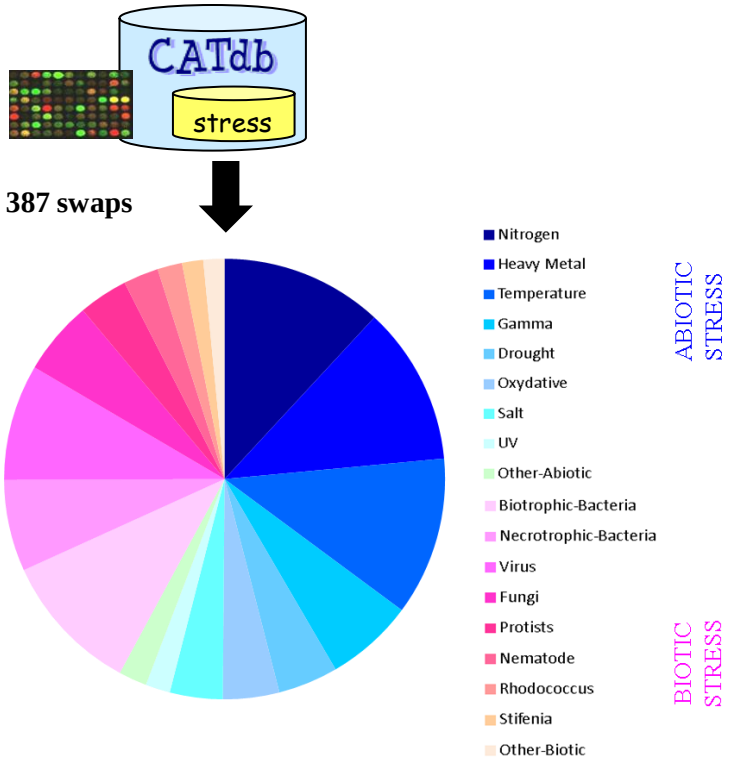
Transcriptomic Dataset

CATdb :Complete Arabidopsis transcriptome database

- Large and homogeneous transcriptome resource generated by the CATMA platform of URGV and available in CATdb (http://urgv.evry.inra.fr/CATdb; Gagnot et al., NAR 2008).

- ~ 6000 original genes not present in the commonly used ATH1 Affymetrix DNA chip.

- All experiments dedicated to stresses were considered: 9 biotic and 9 abiotic stress categories



Differential expression analysis → **17 264** genes have transcription 'impacted' (directly or not) by at least one stress experiment

CoExpression Analysis

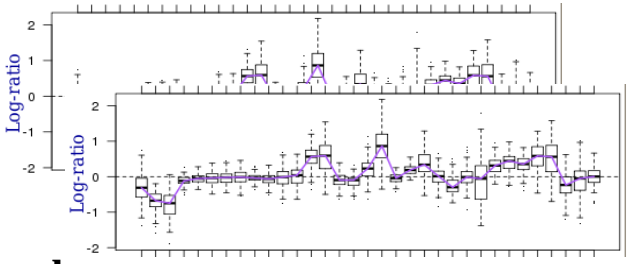
Gene Clustering: Identification of co-expressed genes from the expression differences through a Model based clustering method for each stress category.

Matrix
{ genes x experiments}
By stress

Gaussian Mixture Model

Mathematical Criterion to select the cluster number (BIC)

Classification rule based on conditional probabilities

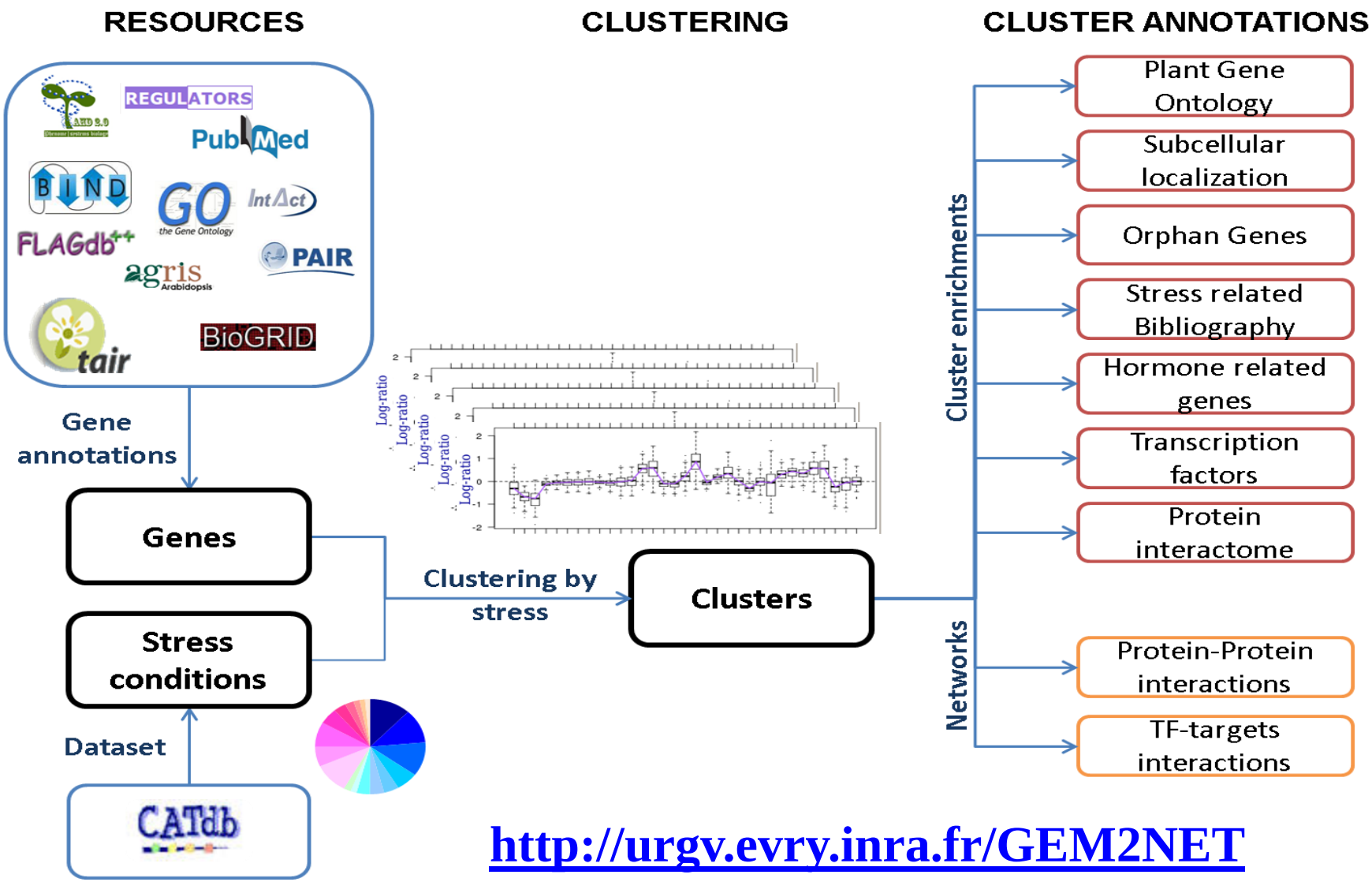


~700 Clusters of Coexpressed Genes

Stress category	Gene_nb	Clusters_nb
Nitrogen	13 495	59
Temperature	11 365	34
Drought	8 143	34
Salt	5 729	30
Heavy metal	10 617	57
UV	7 894	37
Gamma	5 350	32
Oxydative stress	10 127	52
Nectrophic bacteria	11 220	50
Biotrophic bacteria	12 023	56
Fungi	9 773	51
Rhodococcus	1 900	13
Oomycete	5 508	31
Nematode	7 413	27
Stifenia	1 525	17
Virus	11 832	54

GEM2Net Flowchart:

This project has been implemented as a new CATdb module: GEM2Net associated with a user-friendly Interface

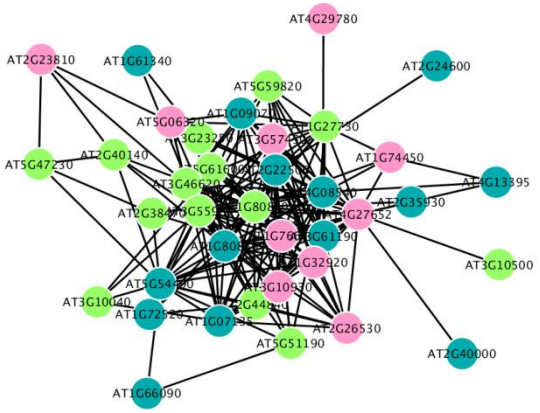


http://urgv.evry.inra.fr/GEM2NET

Functional inference by coregulation analysis

CoExpression clusters for each category of stress

Integration



CoRegulation Network

Identification of Coregulated genes → **Describe groups of functional partners** → **Annotation of orphan genes**

CoRegulation Analysis

What is CoRegulation?

Group of genes which are coexpressed in several stress conditions : key players of stress response

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Occurrence of pairs of coexpressed genes conserved in several stresses among the 18 considered stress categories

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Pairs conserved in at least n stresses

n	Nbr pair of genes
2	5 533 013
3	423 771
4	68 875
5	19 113
6	6 987
7	3 366
8	1 679
9	786
10	324
11	171
12	81
13	39
14	12
15	6

CoRegulation Analysis

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When coexpression becomes coregulation?

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CoRegulation Analysis

➢ We searched a threshold k at which the probability that a pair of genes is coexpressed in k stresses is significantly different from random.

➢ Permutation scheme:

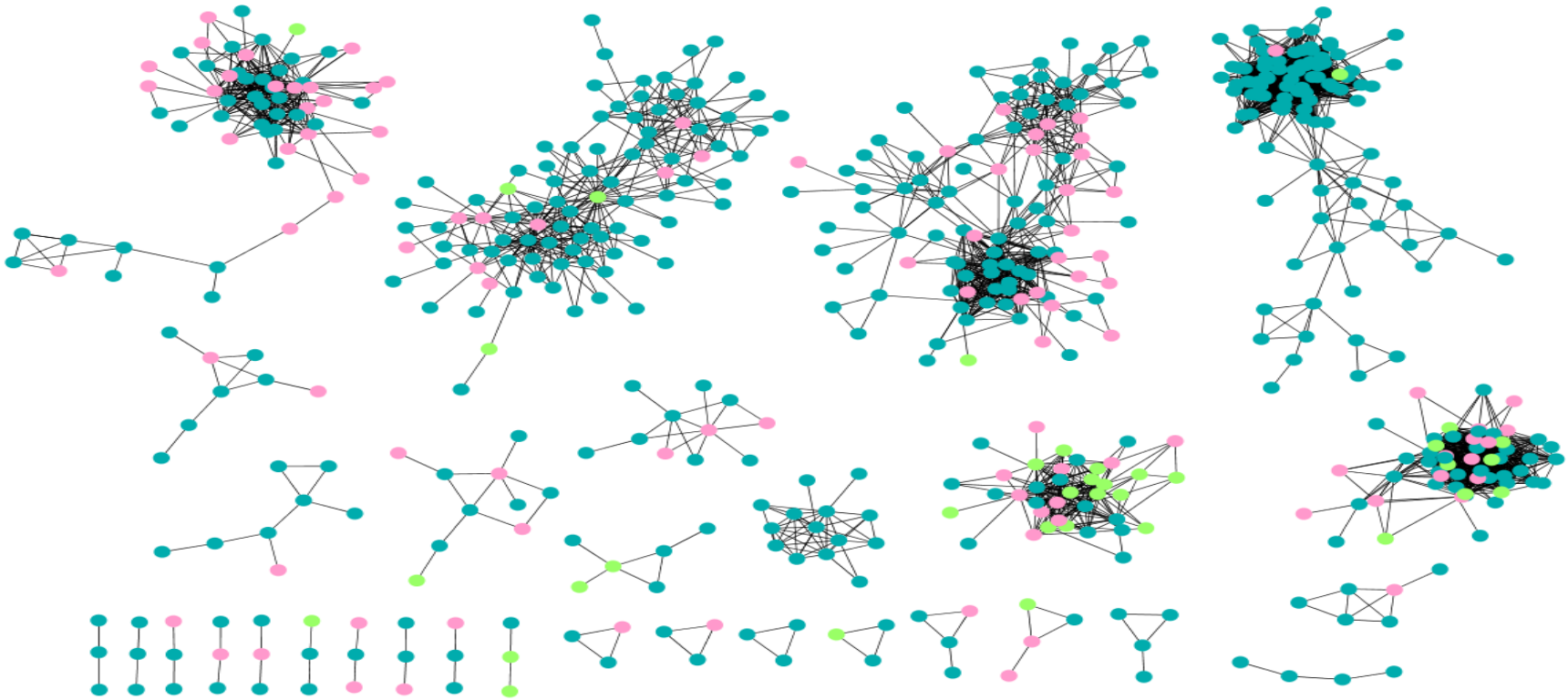
1. Do 1000 times
    a) Shuffle gene classification within each stress category.
    b) Occurrence calculation
2. Error rate calculation: average of occurrence in random samples divided by the occurrence in our data.

Number of stresses (n)	Random Network	Biological Network	Error_rate
4+	1 549	32 313	4.79%
5+	12	13 200	0.09%
6+	0	6 216	0%
7+	0	3 366	0%

Occurrence of pairs conserved in at least n stresses within our random and biological networks

CoRegulation Networks

Pairs conserved in at least 7 stresses
867 genes, 3366 pairs



Legend

- Coregulated genes
- Orphan genes
- TF

CoRegulation Networks

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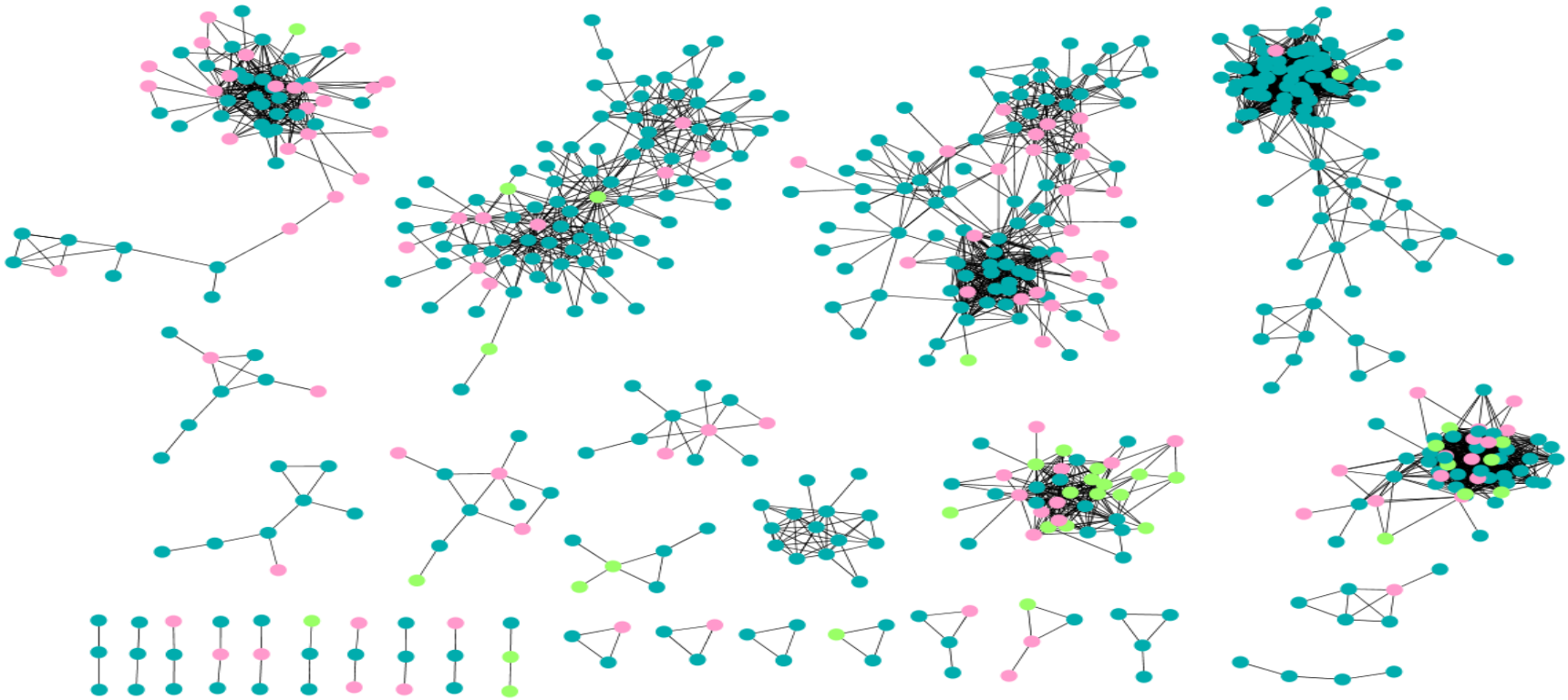
178 Orphans
57 TF



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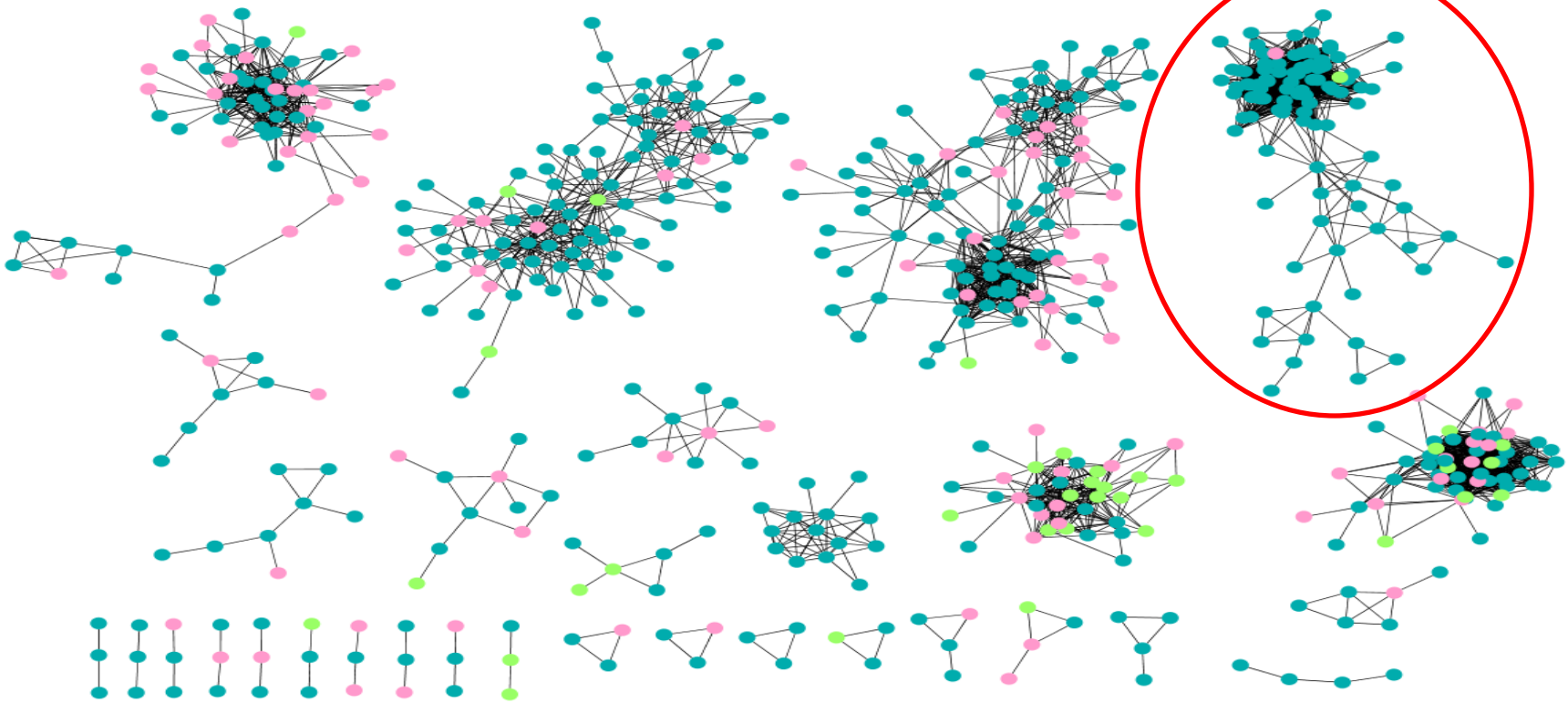

31 connected components

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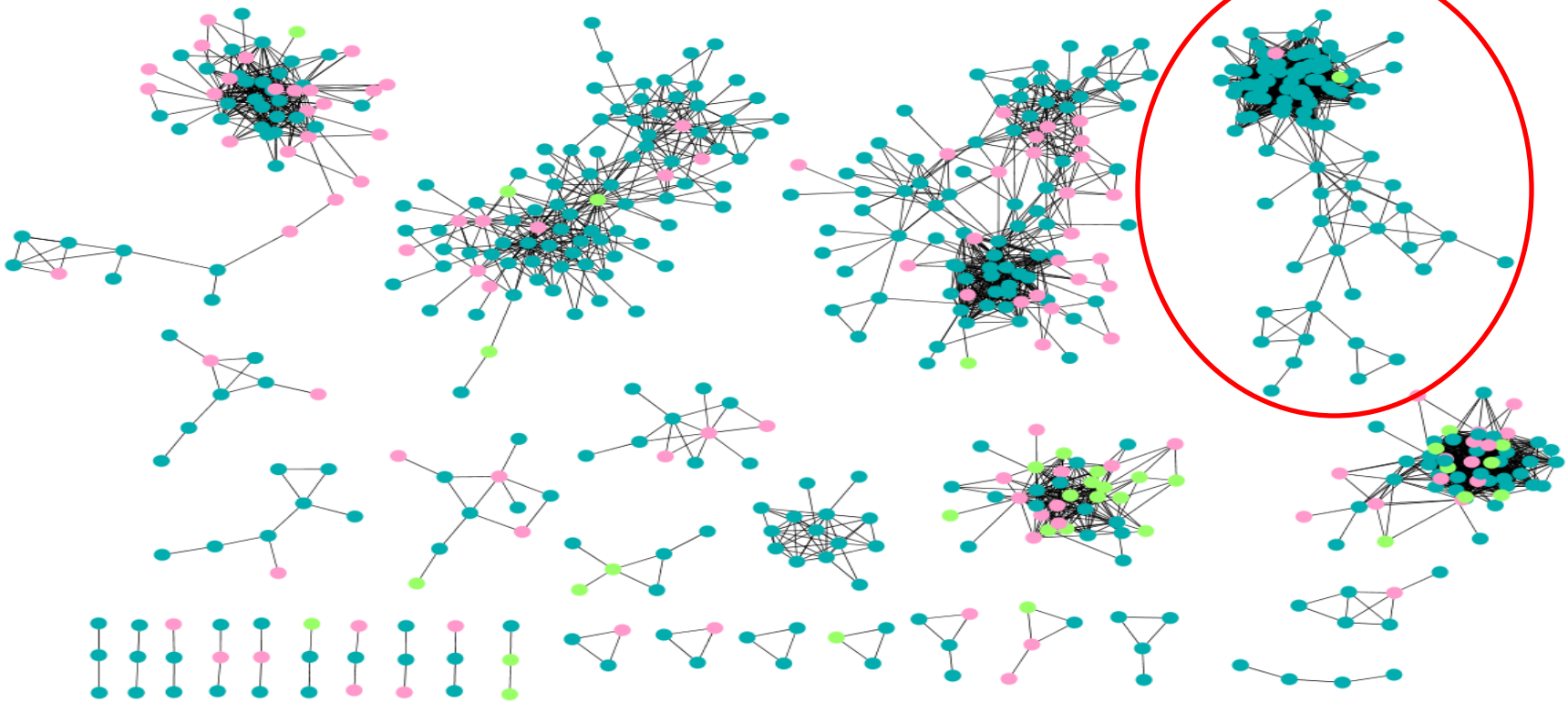

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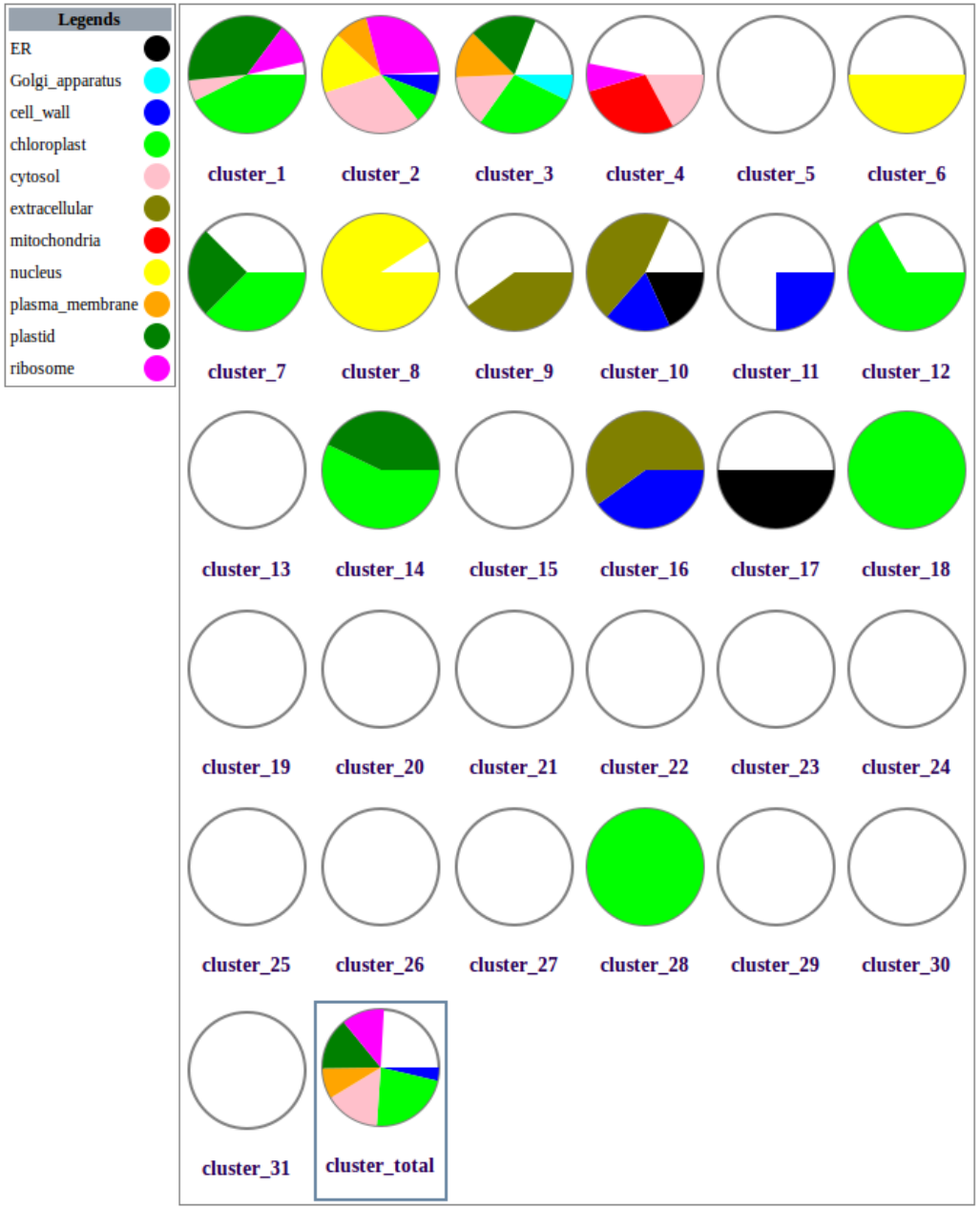
31 connected components
Functional Modules ???

Gene Ontology Enrichment Analysis

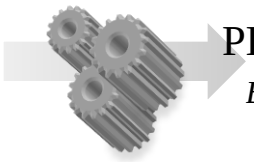


GO:Cellular Component Ontology

Specific and homogeneous modules

Cis-regulatory motifs Enrichment Analysis

Global CoRegulation Network Analysis
(867 genes)



PLMDETECT
Bernard et al., 2010

- 30 TFBS are found Over-represented by comparison with the whole genome present at most in 30% of promoters.

CoRegulation Network Analysis by component
(9 largest components)

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- 4 components are enriched with a pattern that is present in over 60% of their promoters

Cis-regulatory motifs Enrichment Analysis

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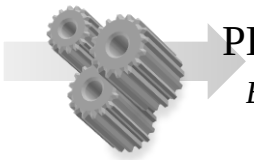

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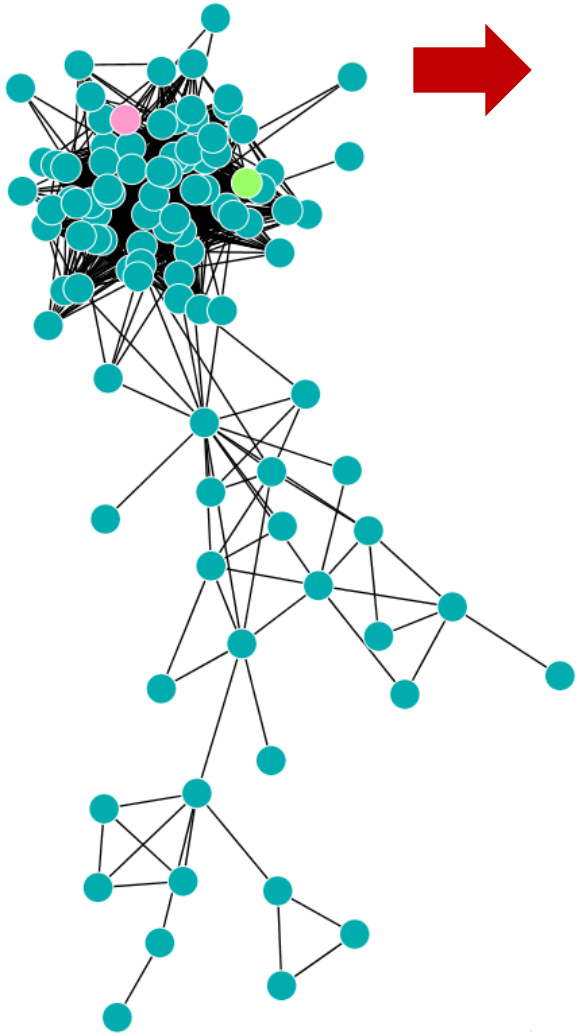
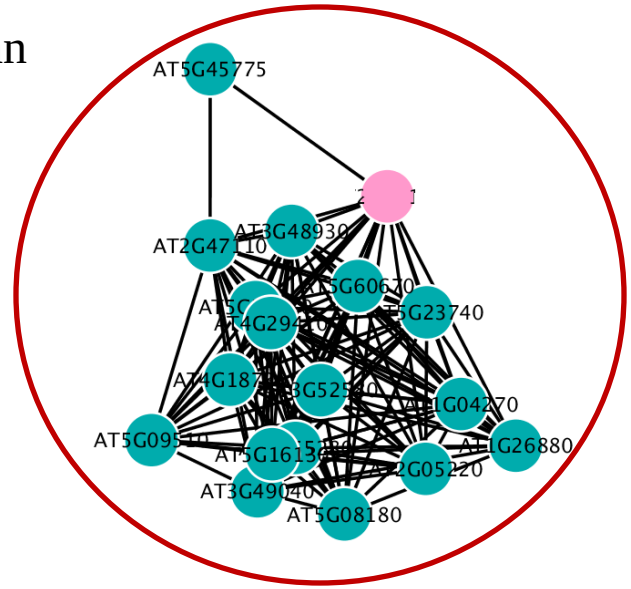
- **Genes are under the control of the same common regulators**
- **Using the network topology is a good track for identifying modules**
- **Module = Functional partners**

Example of an orphan identification within a module

98 genes, 1112 pairs, 1 orphan





First Neighbors of the orphan gene

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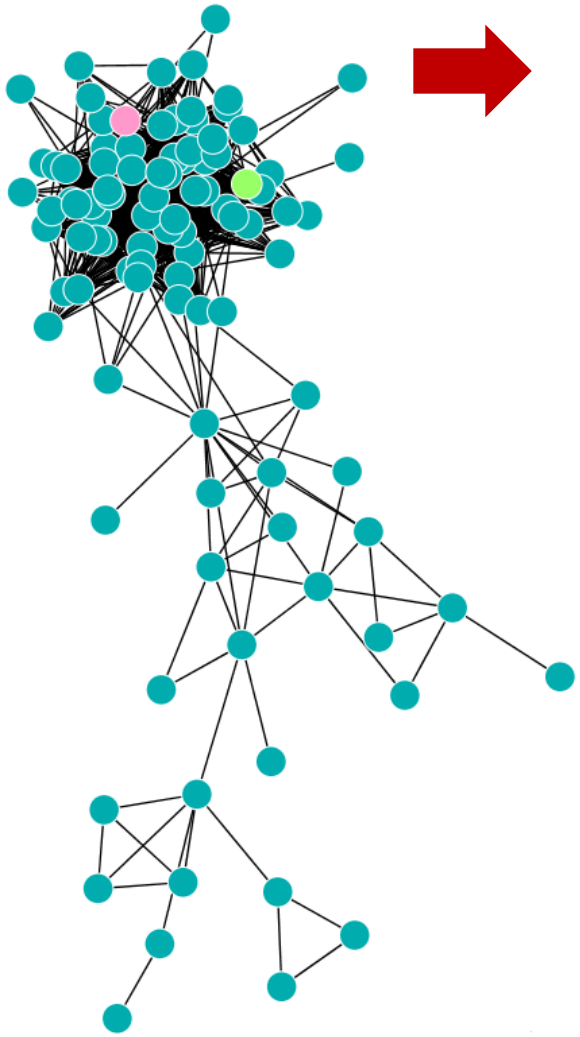
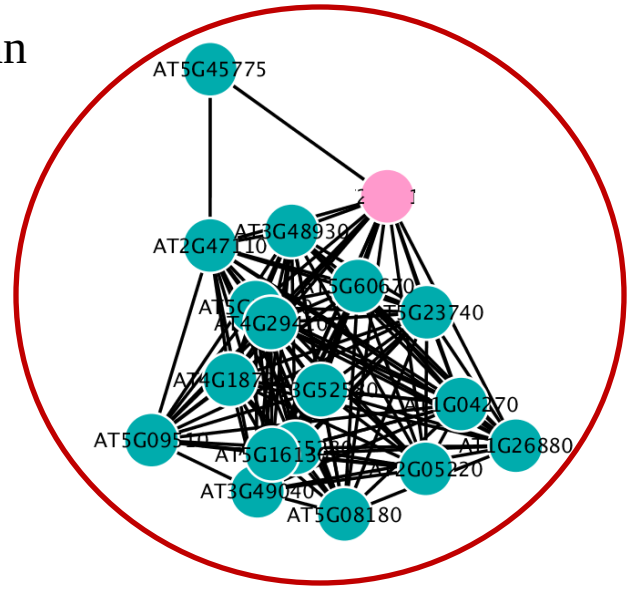
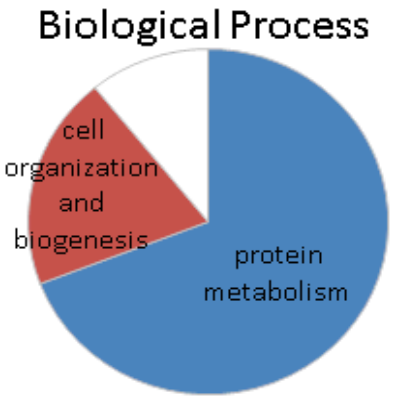
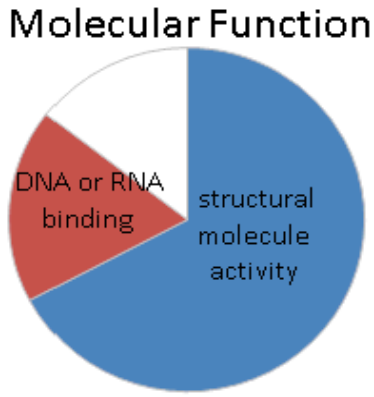




First Neighbors of the orphan gene

15 of the 17 neighbors are annotated as « Structural constituent of ribosome »





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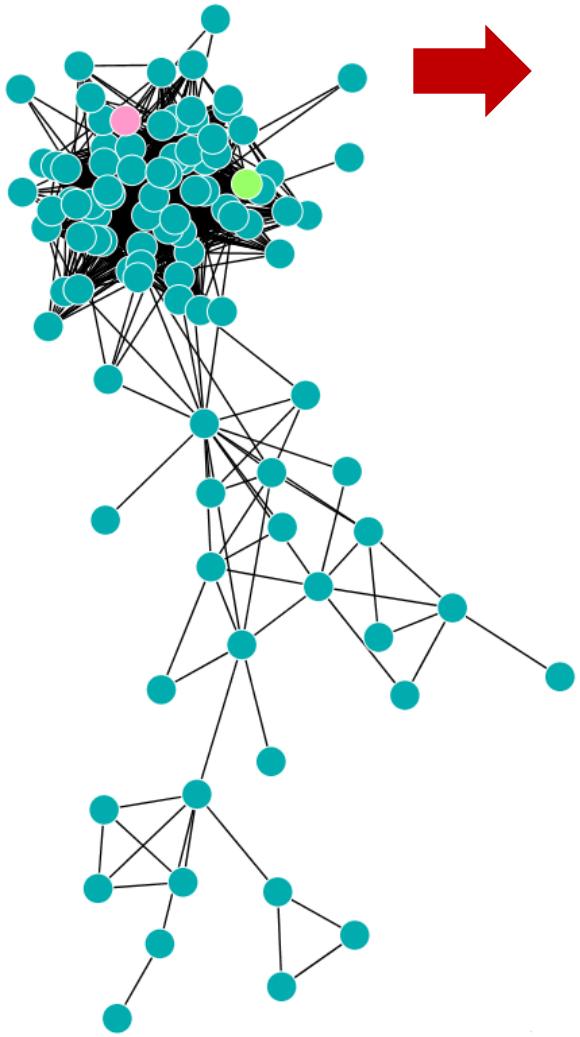

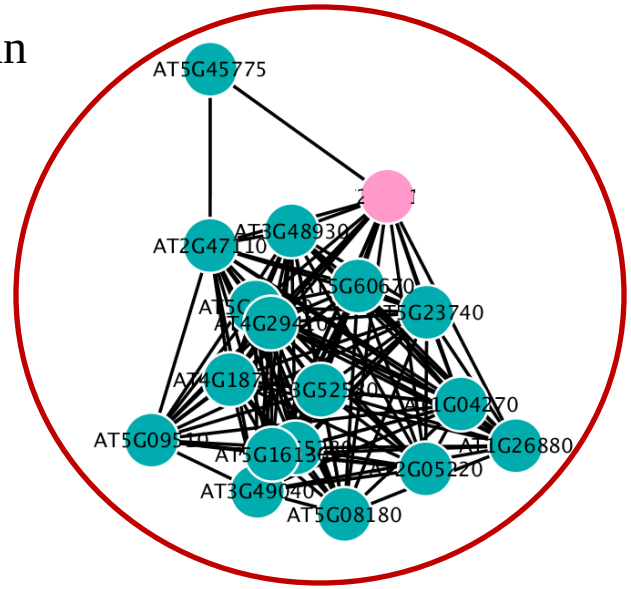

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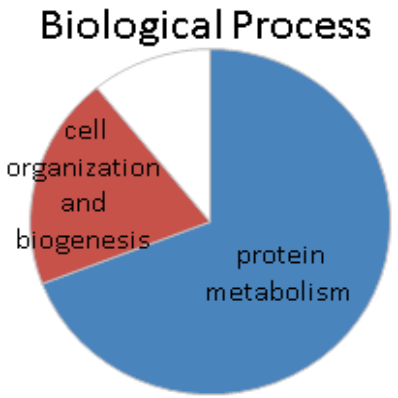

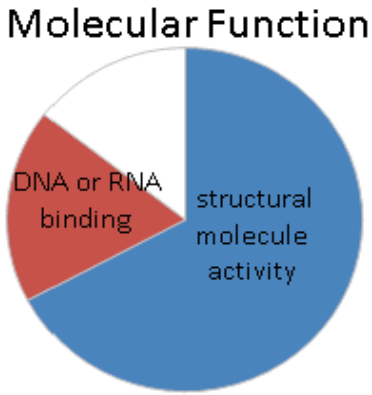

15 of the 17 neighbors are annotated as « Structural constituent of ribosome »

This orphan gene most likely codes for a ribosomal protein

Conclusion & Prospects

At Network « **7+** »
stresses scale

Conclusion & Prospects

At Network « **4+** »
stresses scale

Conclusion & Prospects

At Network « **4+** »
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Conclusion & Prospects

At Network « **4+** »
stresses scale

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Topolgical Network Analysis Method

✓ Refine the search of functional modules using methods of topological network analysis

✓ Integration with interactome data to improve the quality of function inference

✓ First step toward regulatory networks

Acknowledgements

URGV

Bioinformatics

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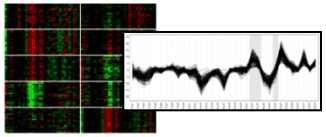
Model-based clustering



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Transcriptome, mutants and stress

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INRA AgroParisTech cnrs

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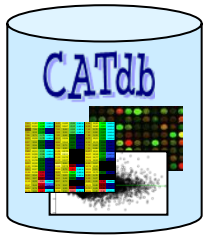
GAP
BV
MIA

Thank you for your attention

Next steps : From clusters to gene networks

From co-regulation clusters to groups of putative functional partners
- Merging and extension of clusters (functions, profiles),
- Experimental step (validation): Transcriptomes of mutants (putative regulators and TF will be targeted)

Transcriptome platform

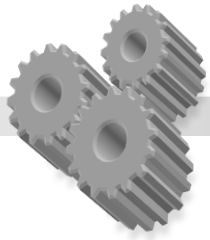


Putative functional partners

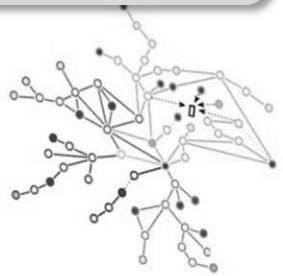
experiment selection

Gaussian Graphical Model

MIA INRA université évry val-d'essonne



Giraud et al. (2009)



Prediction of gene networks (conditional dependencies)

New candidate genes for crop improvement

Translational research

TILLing platform